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Post-translational protein transport by the Sec complex

William J. Allen¹, Ian Collinson¹, Karin Römisch^{2*},

¹ School of Biochemistry, University of Bristol, Bristol, UK

² Faculty of Natural Sciences and Technology, Saarland University, Saarbruecken, Germany

* Correspondence: k.roemisch@mx.uni-saarland.de

Abstract

Although it has been studied for 30 years, the mechanism by which secretory proteins are transported post-translationally into the endoplasmic reticulum (ER) has not yet been fully resolved. Recent structures of the heptameric Secretory (Sec) complex which mediates post-translational import into the yeast ER shed new light on the process.

Many proteins must enter and cross biological membranes to reach their final destination. A primary route for this is through conserved hetero-trimeric channel complexes: Sec61 in the endoplasmic reticulum (ER) of eukaryotes (Sec61 α , Sec61 β , Sec61 γ in mammals; Sec61, Sbh1, Sss1 in yeast); also found in the plasma membrane of bacteria (SecYEG) and archaea (SecYE β). Protein transport can occur either co- or post-translationally. During co-translational transport, N-terminal, hydrophobic signal sequences are recognised by signal recognition particle (SRP) as they emerge from the ribosome and are targeted to the Sec machinery. Structures of the channel have been captured in idle, ribosome-bound, and actively transporting states, giving insights into the structural basis for co-translational secretory protein transport [1].

Additional components, specific to different systems, are required to facilitate post-translational protein transport, and they vary considerably between bacteria and eukaryotes. Structural biology of the bacterial post-translational system by Rapoport and colleagues [2,3], has inspired a renaissance in the translocation field, including recent studies from the authors' laboratories [4,5]. In yeast, for post-translational import into the ER, the Sec61 channel cooperates with the hetero-tetrameric Sec63 complex (Sec62, Sec63, Sec71, Sec72) which binds directly to the Sec61 channel [6-9]. The fungal-specific subunits Sec71 and Sec72 serve as a receptor for post-translational import substrates (pre-proteins) bound to cytosolic Hsp70 [8]. On the ER lumenal side, the J-domain of Sec63 activates the ATPase of the Hsp70 BiP which binds to secretory proteins as they enter the ER and promotes folding [9]. Two new structures of the hetero-heptameric Sec complex (Sec61 complex plus Sec63 complex) now reveal the overall architecture of the post-translationally proficient secretion machinery, and shed fresh light on the mechanism of protein import into the yeast ER [6,7].

Both structures were determined in detergent solution by [cryo-electron microscopy](#) [6,7].

Itskanov [and](#) Park fused the gene encoding the β -subunit of the Sec61 complex (*SBH1*) to the N-terminus of *SEC63-TEV-GFP*. This fusion was then expressed in the presence of wildtype *SEC63*, and the Sec complex purified using the GFP-tag followed by TEV cleavage [6]. Wu *et al.* expressed C-terminally FLAG-tagged *SEC63* as sole copy of the gene, thus ensuring functionality, and purified the native complex via the tag. In addition, they modified surface lysines with polyethylene glycol to randomize its orientation on the grid and prevent aggregation [7].

Overall, the two structures are very similar (Fig. 1a). The main interface between the Sec61 and Sec63 complexes is through Sec63, which interacts extensively with Sec61 in the cytosol, within the membrane, and in the ER lumen [6,7]. Sec62 and the luminal J-domain of Sec63 were not sufficiently well resolved to model in either structure, suggesting that they are both mobile. Weak electron density and the positions of transmembrane helices (TMs) 2 and 3 of Sec63, however, allow them to be positioned approximately [6,7] (Figure 1a).

On the cytosolic side, Sec63 binds to loops 6 and 8 of Sec61 – the same place that the ribosome binds during co-translational transport (Fig. 1a) [1,6,7]. The cytosolic domains of Sec71 and Sec72 sit adjacent to the Sec63 cytosolic domain, sandwiching it in place. Upon binding of a pre-protein/Hsp70 complex to Sec72, this arrangement would position the pre-protein directly at the entrance to the channel through Sec61 [6-9]. While this is perfect for post-translational import, it means that binding of Sec63 and the ribosome to Sec61 are mutually exclusive. Therefore, these structures do not explain the contribution of Sec63 to cotranslational import in both yeast and mammals, unless the ribosome lifts off the channel periodically as suggested by Wu *et al.* [7].

While at rest, the channel formed by the Sec61 complex is both closed and blocked on the luminal side by a plug. In the bacterial system, transport is initiated by binding of the signal sequence into a 'lateral gate' between TMs 2 and 7 of SecY. The movement required for signal sequence insertion occurs at a 'hinge' between TMs 5 and 6, braced by the equivalent of Sss1 (SecE), and opens the channel for pre-protein transport. The new structures reveal that Sec63 binds near the hinge – with TM3 making functionally important interactions with Sss1, Sbh1 and TM1 of Sec61 that span the membrane (Fig. 1b) [6-7]. Using site-directed mutagenesis, Wu *et al.* showed that Sec63 residues that contact Sec61 within the membrane and at the cytosolic surface, but not at the ER luminal face, are essential for post-translational import into the ER [7].

Binding of Sec63 induces an opening movement in Sec61 similar to that seen in the activated bacterial SecY (Fig. 1c). In the Wu *et al.* structure, the cytosolic end of the lateral gate is wide open, while the luminal end remains partially closed, with the plug still in place [7] (Fig. 1b-c, blue/green). In the Itskanov and Park structure, on the other hand, the cytosolic end of the lateral gate is more closed, but the luminal end is completely open, and the plug unresolved [6] (Fig. 1b, red/yellow). This difference could be due to the stabilising influence of the N-terminus of Sec63 (present only in the Wu *et al.* structure), or differential loss of stabilising lipids due to use of different detergents during purification. Either way, in the Sec complex, the lateral gate and channel are clearly poised ready to accept the signal sequence of an incoming pre-protein. As both papers observe, this is likely a requirement for insertion of the weaker, less hydrophobic signal sequences that characterise post-translational translocation [6,7]. Indeed, ribosome binding to Sec61 for co-translational import does not induce the same change (Fig. 1c, magenta). This is reminiscent of the bacterial post-translational translocation system, which also accept less hydrophobic signal sequences compared to co-translationally

translocated substrates [10]; in bacteria, the post-translational partner SecA helps to prise open the lateral gate of SecY [2].

Overall, these structures illuminate how the post-translational ER import machinery is put together, and provide plausible mechanisms for its role in the recruitment, initiation, and transport of pre-protein substrates. And as other protein transport complexes have shown, the availability of structures is a vital aspect of understanding broader aspects of control and function.

Figure 1: Sec complex structures. a) Side views of the new Sec61-Sec63 complex structures from the [Park](#) lab [6] (left; PDB [6N3Q](#)) and the [Rapoport](#) lab [7] (right; PDB [6ND1](#)). b) Luminal view of the trans-membrane helices from the [two new Sec61-Sec63 structures](#), [coloured using the](#) same colour scheme as above. The structures were superimposed over the N-terminal half (TMs 1-5), [and overlay very well, except for in the tilt of the lateral gate helices TM2 and TM7](#). c) Same view as in panel b, but comparing the [Wu *et al.* structure](#) to the previous ribosome-bound mammalian Sec61 structure [1] (PDB 3J7Q) (with the α , β , and γ subunits coloured magenta, purple and pink, respectively). [These structures are also superimposed over TMs 1-5, illustrating the opening of the lateral gate induced by Sec63.](#)

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